

A Correlation Study of Organochlorine Levels in Serum, Breast Adipose Tissue, and Gluteal Adipose Tissue among Breast Cancer Cases in India

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Abstract

We used data from a breast cancer pilot study carried out in Kerala, India in 1997, for which organochlorine levels were measured in three biological media, blood serum, breast adipose tissue, and gluteal adipose tissue, of 37 fasting breast cancer cases (pretreatment). Our objective was to investigate the relationships between organochlorine concentrations in different biological media. Gas-liquid chromatography determined serum, breast adipose, and gluteal adipose tissue levels of dichlorodiphenyltrichloroethane, dichlorodiphenyl-dichloroethane, β -benzene hexachloride, and polychlorinated biphenyl (PCB) congeners, PCB-153 and PCB-180. Correlation plots were made and Spearman correlation coefficients (r) calculated for breast adipose tissue versus serum, gluteal adipose tissue versus serum, and breast adipose versus gluteal adipose tissue. We also examined paired ratios of all summary statistics. There were strong correlations among serum, breast adipose tissue, and gluteal adipose tissue concentrations for most organochlorines analyzed, one exception being gluteal

versus serum for PCB-153. The correlations for all other comparisons ranged from $r = 0.65$ to 0.94 . Serum (ng/g) versus adipose ratios approached 1:1 for most of the organochlorine pesticide comparisons and did not vary by summary statistic. To our knowledge, this is the first study to use three different media from fasting subjects and to comprehensively investigate the relationship between organochlorines measured across the three media for both organochlorine pesticides and PCBs. These data indicate that blood serum reflects the present body burden of a range of organochlorines to the same extent as adipose tissue, and they support the view that serum may be collected in lieu of adipose tissue to obtain similar information. However, such measurements are a combination of both recent exposures and past exposures, which have metabolized slowly and may still persist. Therefore, investigators should use caution when assigning a level as lifetime body burden. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1113–24)

Introduction

The steady-state partitioning of lipophilic compounds, such as organochlorines, between blood serum and other media, such as breast adipose tissue and subcutaneous fat, is an important consideration when trying to assess the body burden of these chemicals from long-term exposure. If concentrations in different biological media (serum and fat tissue) are highly correlated, then measurement in either may be considered a reliable biomarker of exposure. Whereas biopsy is a viable way to obtain breast adipose tissue on patients with suspected breast disease, this is not an option for healthy subjects, but blood serum is a biological material that can be obtained conveniently and in large populations. It is still uncertain whether serum levels of organochlorines reflect the body burden of these chemicals.

A series of articles were published in the 1990s (1–5), comparing levels of particular organochlorines detected in blood serum with levels of the same organochlorines in other media in paired samples; results were conflicting. Three (1, 2, 5) of these five studies concluded that either serum or

adipose levels could be used as biomarkers for lifetime body burden and that the correlation between the two media were high; however, two of the studies recommended that adipose tissue be analyzed in addition to serum, to fully understand human exposure to organochlorines (3, 4). One study did not make any specific conclusions about the use of serum in lieu of adipose tissue (5). Only two of the studies measured levels in fasting women (3, 4), and only one (2) included a range of both organochlorine pesticides and polychlorinated biphenyl (PCB) congeners. Additionally, only two studies expressed results for serum, both uncorrected for (wet weight basis) and corrected for lipids (1, 2). A recent study of 21 breast cancer cases who provided a sample of both breast adipose tissue and abdominal adipose tissue investigated agreement and correlation between paired measurements of organochlorine pesticides, polychlorinated biphenyls (PCB), polybrominated diphenyl esters, and dioxins. The authors found high correlations between the two media for most of the target chemicals (6). They did not include serum in their study and commented that measurements in serum may be biased because of fluctuations of lipids and because of analytic errors adding to the discrepancy between measurements in adipose and blood (6).

In reviewing the literature, we noted that a study among fasting subjects, for whom measurements were taken in multiple biological media for a range of organochlorine pesticides and PCB congeners, and for whom correlation coefficients and ratios were calculated for both serum on both

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a wet weight and lipid-adjusted basis, had not previously been carried out. We therefore analyzed data on organochlorine levels in three biological media, blood serum, breast adipose tissue, and gluteal adipose tissue, from a pilot study carried out in 1997, which was initiated to support the feasibility of a proposed breast cancer case-control study in Kerala, India. The organochlorines we investigated included three pesticides, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDE), and β -benzene hexachloride (β -HCH) and two of the most abundant PCB congeners, PCB-153 and PCB-180 (2). Our objective was to comprehensively investigate the relationships between organochlorine levels detected in different biological media by taking advantage of these existing samples and carrying out analyses based on calculations of correlations and paired ratios of three biological measurements in each woman.

To our knowledge, this is the first study to evaluate both the correlations and ratios of organochlorine concentrations in blood serum, breast adipose tissue, and gluteal adipose tissue in fasting women. This is an interesting population to study with respect to organochlorine pesticides, because some of them are still commonly used in India. Although there has been a ban on both DDT and β -HCH in most industrialized countries for about 30 years, India still continued to import and use both to control malaria-spreading mosquitoes until recently (7). Because this is a rural population, there is likely low exposure to industrial agents; thus, we expected PCB levels to be lower than those measured in previous studies of organochlorine partitioning in biological media (1-4).

Materials and Methods

Study Population

Study subjects were recruited from women who underwent breast surgery for newly diagnosed breast cancer at the Regional Cancer Center, Trivandrum, Kerala, India, between February 1, 1996 and January 31, 1997. Breast cancer case status was ascertained pathologically at the Regional Cancer Center. Written informed consent was obtained from all participants, and the study was approved by the institutional review boards of the Regional Cancer Center and the National Cancer Institute. Patients were eligible for the study if they had no previous diagnosis of breast cancer, no history of any other cancer (excluding nonmelanoma skin cancer), no history of treatment for breast cancer (except for biopsy in the past 2 months), were residents of Kerala, and were scheduled for breast surgery at the Regional Cancer Center. Patients who met eligibility requirements were identified by hospital surgeons and referred to the study interviewer for an in-person interview. Fasting blood samples ($n = 35$) were collected just before breast surgery, and breast ($n = 34$) and gluteal ($n = 33$) adipose tissue samples were obtained at the time of surgery. Each patient's age and stage at diagnosis were abstracted from medical records.

Laboratory Analyses

Blood samples were stored in a refrigerator; separated within 12 hours into plasma, buffy coat, and RBC; and subsequently stored at -80°C . Breast and gluteal adipose tissue samples were collected into glass vials, coded and frozen within 30 minutes of being excised, and stored at -80°C . All three specimen types were obtained from 37 women. Frozen serum and adipose samples were sent in batches to the United States to the Research Triangle Institute International, where they remained frozen until analysis. Samples were analyzed in batches of eight, with each batch having five to six cases and two to three quality control samples. There was quality control data for the serum measurements but not for breast or gluteal adipose tissue.

Both intrabatch and interbatch coefficients of variation (CV) were calculated. Gas chromatography with electron capture was used to measure organochlorine levels. Lipid-adjusted values of organochlorines were calculated by dividing the tissue and serum levels of organochlorines by the total lipid value in each sample.

Assay Methods for DDE, DDT, β -HCH, and PCBs

Serum. One milliliter of thawed serum was transferred to a centrifuge tube and the volume of the serum recorded to ± 0.01 mL. Ten nanograms of a surrogate PCB (PCB-198) were added to the serum. One milliliter of methanol was added to denature the albumin. The mixture was extracted thrice with 5 mL of 50% ethyl ether (*n*-hexane). The extracts combined and concentrated to 10.0 mL. Two milliliters of this extract were removed for lipid determination. The remaining 8 mL were concentrated to 1 mL and transferred to 1 g of Florisil (deactivated before use with water). The SPE column and the pesticides and PCBs were eluted with 25 mL *n*-hexane (fraction 1), followed by 25 mL of 10% ethyl ether in *n*-hexane (fraction 2). The first elute contained DDE and other nonpolar pesticides. The fractions were concentrated to 1.0 mL, added to 10 ng internal standard (PCB-119), and analyzed by gas chromatography with electron capture detection. The gas chromatography with electron capture detection was calibrated by repetitive analysis of standard mixtures of selected individual PCB congeners at six levels for the quantification of individual congeners. The analysis method for individual PCB congeners was a modified version of established methods (8-12).

Adipose tissue. Approximately 100 and 50 mg of breast and gluteal adipose tissues, respectively, were weighed and transferred to a 15-mL culture tube. Ten nanograms of the surrogate PCB were added to the tissue sample. The tissue was dried by grinding with 1 g of anhydrous sodium sulfate. The dried sample was extracted thrice with 5-mL portions of hexane. The extracts were combined and the extract volume adjusted to 15 mL. Two milliliters of this extract were removed for lipid determination, whereas the remaining 13 mL were reduced to 1 mL. The extract was transferred to 1 g of Florisil SPE column, and the pesticides and PCBs were eluted as described above for sera extracts.

For 1 mL of sera, the limit of detection was 0.01 ng/g for DDE, DDT, and β -HCH and 0.005 ng/g for individual PCBs congeners. For 100 mg of breast adipose tissue and 50 mg of gluteal adipose tissue, the limit of detection was 0.1 ng/g for DDE and DDT and 0.05 ng/g for PCBs. All measurements for the organochlorines presented in this study were above the limit of detection.

The method for the total lipid analysis was adopted from the methods used by Sheldon (13, 14). We calculated lipid-adjusted serum values by dividing serum levels of the organochlorines of interest by the total lipid value, as determined from the methods discussed by Philips et al. (15).

Statistical Analyses

Thirty-seven fasting breast cancer patients (with no prior treatment) in the district of Kerala, India each provided at least two biological media. A total of 35 provided serum, 34 provided breast adipose tissue, and 33 provided gluteal adipose tissue. There were 21 women for whom all three biological media were provided; however, because we carried out correlations between two media at a time, we included women who provided both (a) serum and breast adipose tissue, $n = 25$; (b) serum and gluteal adipose tissue, $n = 24$; and (c) breast and gluteal adipose tissues, $n = 29$.

Arithmetic means, medians, geometric means, and ranges (minimum, maximum) were calculated for DDT, DDE, β -HCH, PCB-153, and PCB-180 concentrations in blood serum (both lipid-adjusted and, for purposes of comparison

with previous studies, non-lipid adjusted), breast adipose tissue, and gluteal adipose tissue. To investigate the steady-state partitioning of the organochlorines measured, correlation plots were made and Spearman correlation coefficients (r) were calculated for (a) breast adipose tissue (ng/g) versus serum (ng/mL), (b) breast adipose tissue (ng/g) versus lipid-adjusted serum (ng/g lipid), (c) gluteal adipose tissue (ng/g) versus serum (ng/mL), (d) gluteal adipose tissue (ng/g) versus lipid-adjusted serum (ng/g lipid), and (e) breast adipose tissue (ng/g) versus gluteal adipose tissue (ng/g). We chose Spearman correlation coefficients because data were not normally distributed. Ratios of these five relationships were also calculated and are presented.

Results

Women in this population were between 24 and 65 years of age. This was a fairly parous population, in that 80% of the women in this study had experienced at least two live births and 40% had at least three live births. Women in this study breast-fed for a relatively long time; the mean cumulative number of months a woman in this study breast-fed was 52.6 months. Ten of the women (29%) in this study breast-fed for >69 months (nearly 5 years) cumulative. Among the 14 postmenopausal women, age at menopause ranged from 26 to 54 years and was fairly evenly distributed. Exposure to DDT was likely a result of directly applying it because 29 women (83%) reported ever having used DDT at home or at work and 22 (63%) reported ever having personally applied it.

For serum, we calculated both intrabatch and interbatch variation (batch SD divided by batch mean and sample standard variation divided by sample mean, respectively) and detected that one batch consistently had high intrabatch coefficients of variation (about 60-70%, depending on the organochlorine measured). We therefore excluded the six serum samples that were measured in that batch; this left us with 29 samples with blood serum measurements. After excluding that batch, our interbatch CV also improved. The

following interbatch CVs were found for blood serum: $DDE_{CV} = 19.4\%$, $DDT_{CV} = 8.0\%$, $\beta\text{-HCH}_{CV} = 33.5\%$, and $PCBs_{CV} = 10.1$. We detected one outlier for gluteal adipose tissue measurements and rejected it as an outlier because it was orders of magnitude lower than the measurements of other gluteal samples; yet the measurement in serum (there was no measurement in breast adipose tissue for this sample) was within the range of other serum samples in the study. Subsequently, the ratio of serum to gluteal adipose tissue for this sample was orders of magnitude higher than the ratios of serum to gluteal adipose tissue for all other samples. This left us with 32 gluteal adipose tissue samples.

Table 1 presents the arithmetic mean, median, geometric mean, and range for DDT, DDE, $\beta\text{-HCH}$, PCB-153, and PCB-180, measured in blood serum [presented on both a wet weight (ng/mL) and lipid corrected (ng/g lipid) basis], breast, and gluteal adipose tissues. The highest concentrations of DDE, PCB-153, and PCB-180 were found in lipid-adjusted blood serum; this was not the case for DDT and $\beta\text{-HCH}$, where concentrations were higher in gluteal and breast adipose tissues than in lipid-adjusted blood serum. We stratified the cases into two groups by stage, to investigate whether levels in all three biological media differed by stage. The first group included cases with stage I or II breast carcinoma; the second group included cases with stage III or IV breast carcinoma. Differences between the two groups were negligible (data not shown).

Because body burden of organochlorines is strongly associated with age, we also stratified by age at diagnosis: younger (≤ 45 years) and older (> 45 years). We found that concentrations of all organochlorines were consistently lower in the younger age group compared with the older age group (data not shown), which is consistent with the persistent properties of these chemicals and their accumulative nature. Breast-feeding is also an important factor to consider because it is a highly efficient way of lowering one's body burden (16). We stratified the population into shorter (≤ 48 months) and longer (> 48 months) time of breast-feeding and found that the women who breast-fed for > 48 months did have lower concentrations than women who breast-fed up to 48 months.

Table 1. Mean, median, geometric mean, and minimum and maximum levels of organochlorines measured in breast tissue, gluteal tissue, and blood serum (both ng/mL and ng/g lipid)

	<i>n</i>	Mean (SD)	Median (25th, 75th percentile)	Geometric mean (95% CI)	Min	Max
DDE						
Serum (ng/mL)	29	4.72 (4.42)	3.11 (2.06, 5.21)	2.82 (2.00-4.01)	0.23	19.90
Serum (ng/g lipid*)	29	944.48 (924.96)	619.44 (446.75, 1,159.65)	678.90 (114.94-4,009.81)	45.8	4,422.00
Breast (ng/g)	34	609.37 (631.77)	421.50 (245.00, 690.00)	417.66 (75.10-2,322.82)	26.70	2,780.00
Gluteal (ng/g)	32	853.14 (840.32)	482.00 (346.00, 1,115.00)	632.78 (125.46-2,515.21)	56.6	4,350.00
DDT						
Serum (ng/mL)	29	0.76 (0.47)	0.47 (0.37, 0.94)	0.57 (0.43-0.75)	0.10	2.94
Serum (ng/g lipid*)	29	149.92 (126.57)	97.36 (75.03, 189.51)	114.97 (31.48-419.91)	20.8	653.30
Breast (ng/g)	34	164.05 (159.04)	101.08 (58.50, 194.00)	113.58 (28.90-446.31)	23.00	623.00
Gluteal (ng/g)	32	225.76 (211.36)	129.00 (93.40, 307.00)	183.25 (45.56-514.16)	44.6	950.00
$\beta\text{-HCH}$						
Serum (ng/mL)	29	14.45 (7.70)	14.20 (9.17, 18.90)	12.45 (6.04-25.66)	2.78	3,840.00
Serum (ng/g lipid*)	29	2,876.15 (1,537.34)	2,818.18 (1,935.06, 3,643.41)	2,506.94 (298.23-21,073.79)	556.00	8,258.06
Breast (ng/g)	34	3,118.85 (1,574.52)	2,675.00 (1,830.00, 3,950.00)	2,751.28 (295.38-25,626.89)	751.00	7,500.00
Gluteal (ng/g)	32	4,631.00 (2,933.00)	4,105.00 (2,680.00, 6,075.00)	3,952.34 (490.00-23,660.22)	786.00	12,640.00
PCB-153						
Serum (ng/mL)	29	0.08 (0.08)	0.06 (0.05, 0.08)	0.07 (0.03-0.15)	0.02	0.39
Serum (ng/g lipid*)	29	16.08 (11.90)	11.39 (9.93, 17.45)	13.40 (6.84-26.24)	3.24	50.91
Breast (ng/g)	34	11.54 (19.14)	5.03 (2.56, 11.90)	4.65 (2.33-9.31)	0.02	102.00
Gluteal (ng/g)	32	10.76 (8.88)	7.43 (5.29, 14.15)	9.12 (3.82-11.73)	0.02	36.60
PCB-180						
Serum (ng/mL)	29	0.03 (0.03)	0.02 (0.02, 0.03)	0.07 (0.03-0.15)	0.01	0.19
Serum (ng/g lipid*)	29	5.81 (3.99)	5.07 (3.78, 6.79)	13.40 (6.84-26.24)	1.96	24.55
Breast (ng/g)	34	4.58 (4.87)	2.86 (1.60, 5.11)	4.65 (2.33-9.31)	0.59	22.90
Gluteal (ng/g)	32	4.67 (4.46)	3.29 (1.44, 5.96)	4.27 (3.82-11.73)	0.40	18.90

*Lipid adjusted.

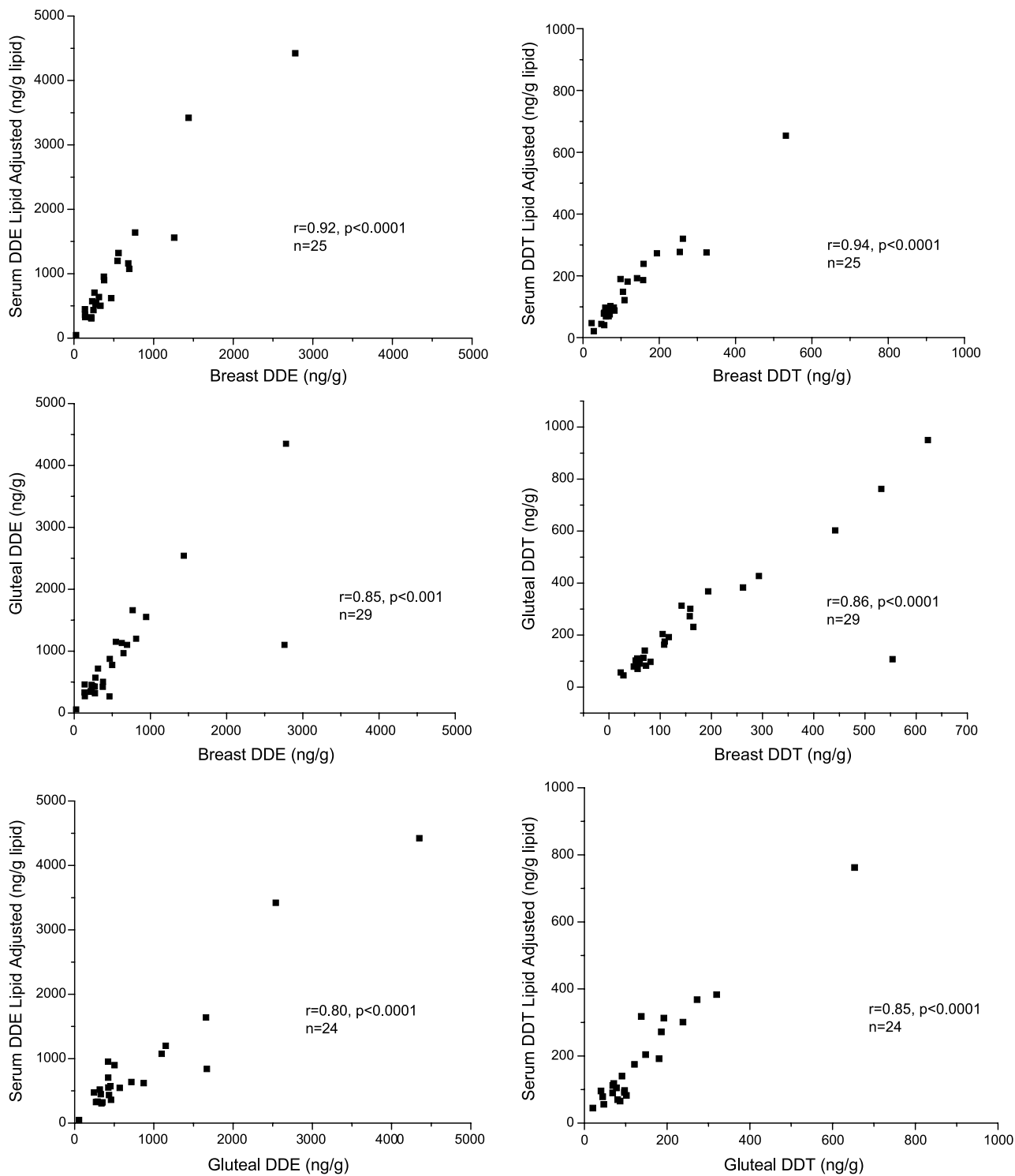


Figure 1. Correlation plots and Spearman correlation coefficients for DDE, DDT, β -HCH, PCB-153, and PCB-180.

It is difficult to compare the literature pertaining to concentrations of organochlorines measured in blood serum, breast adipose tissue, and other media (i.e., breast milk), because inconsistent units have been presented throughout [i.e., some studies use ng/g, others use $\mu\text{g/g}$, others simply present parts per billion (ppb) or parts per million (ppm)], and some authors presented lipid-adjusted measurements for serum, whereas others did not lipid correct. To clarify the findings in the literature and to determine where our study population ranks, with respect to serum and adipose tissue concentrations,

we converted concentrations reported in other studies into ng/mL (for serum), ng/g-lipid (for serum), or ng/g (for adipose tissue). Tables for concentrations of DDE, DDT, β -HCH, PCB-153, and PCB-180 measured in other studies throughout the literature (predominantly literature pertaining to breast cancer) are presented in Appendices 1 to 5, respectively. In each table studies ranked the studies in descending order, based first on the levels in lipid-adjusted serum (the most frequently used measurement throughout the literature) and then on the levels in breast adipose tissue. We present both the year of the study

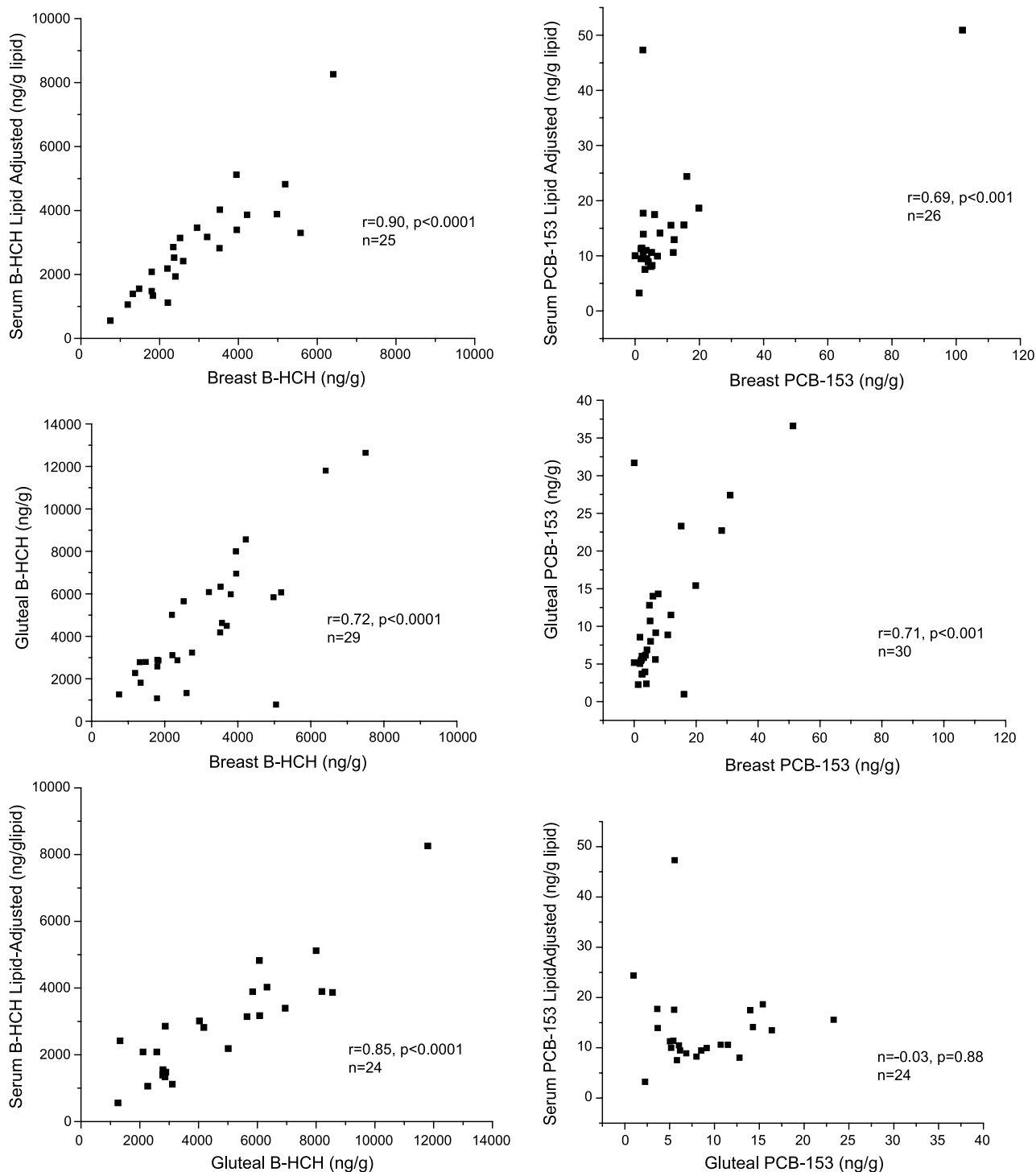


Figure 1. Continued

and the period (year(s)) during which biological samples were collected. The levels of the metabolite, DDE, detected in our study rank somewhere in the middle; however, the levels of DDT were higher than that found in most other studies. There were notably high concentrations of β -HCH detected in this population, orders of magnitude higher than those levels found in all other populations in recently published breast cancer studies. Our study population has the lowest levels of PCB-153 and PCB-180 measured throughout the literature we reviewed.

Correlation plots and Spearman correlation coefficients (r) are presented in Fig. 1 for three comparisons: (a) breast adipose

tissue versus lipid-adjusted serum, (b) gluteal adipose tissue versus lipid-adjusted serum (not including the one sample which was rejected as an outlier), and (c) breast versus gluteal adipose tissue. For all five organochlorine compounds, there were high, statistically significant correlations for these comparisons (range, $r = 0.65$ - 0.94), with the exception of one comparison: gluteal adipose tissue versus serum levels of PCB-153. The strongest correlations were found for DDT and DDE breast adipose tissue versus lipid-adjusted serum comparisons ($r_{DDE} = 0.92$, $r_{DDT} = 0.94$). We compared serum concentrations (ng/mL) with lipid-adjusted serum concentrations (ng/g lipid)

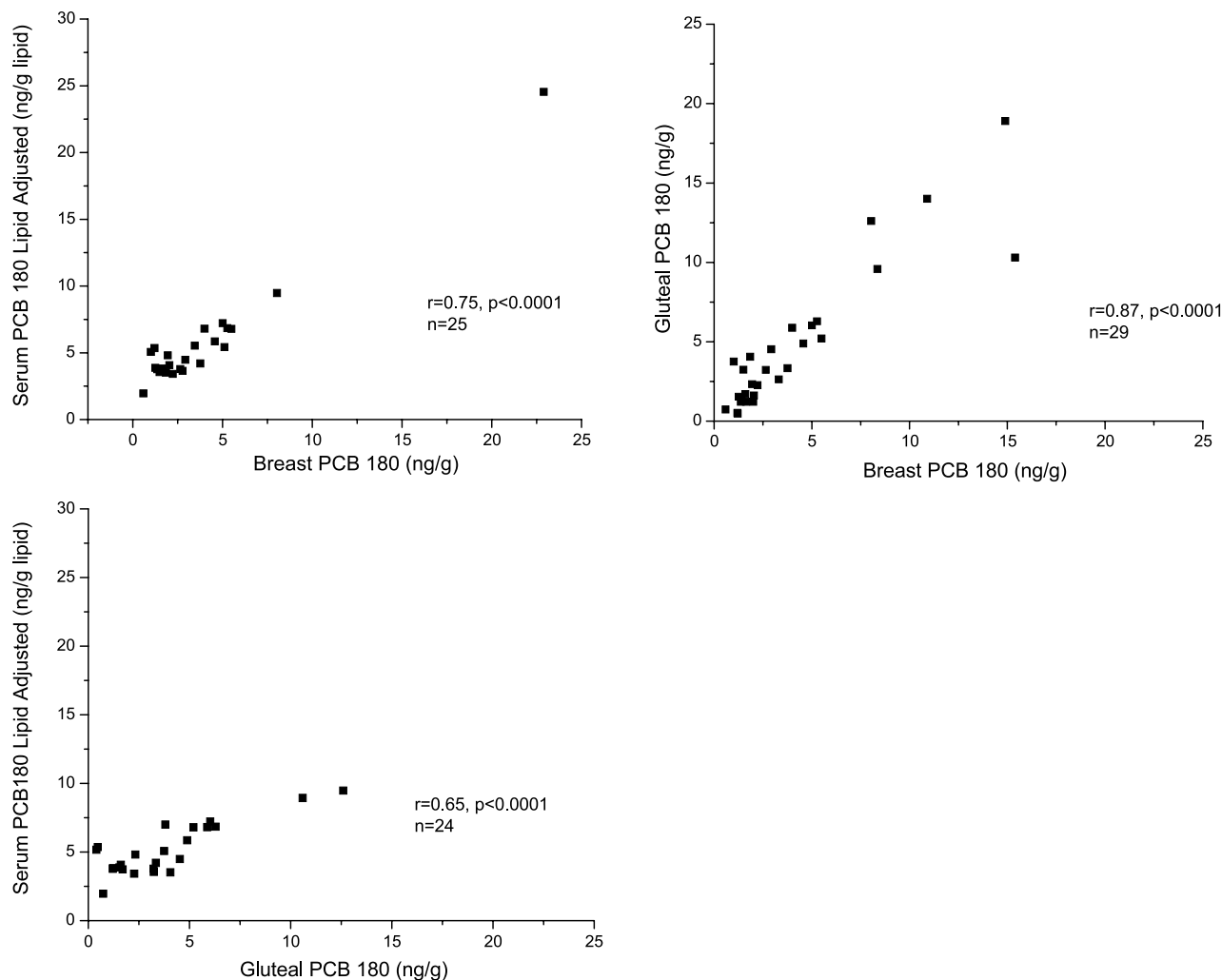


Figure 1. Continued

and found high correlations for all five organochlorines (data not shown), which indicates that the variability of lipids in this fasting population was quite low. Because of these high correlations, we do not present additional plots for breast adipose tissue versus non-lipid-adjusted serum ($r_{\text{DDE}} = 0.94$, $r_{\text{DDT}} = 0.95$, $r_{\beta\text{-HCH}} = 0.85$, $r_{\text{PCB-153}} = 0.85$, $r_{\text{PCB-180}} = 0.77$) or for gluteal adipose tissue versus non-lipid-adjusted serum ($r_{\text{DDE}} = 0.82$, $r_{\text{DDT}} = 0.85$, $r_{\beta\text{-HCH}} = 0.83$, $r_{\text{PCB-180}} = 0.78$), because they are very similar to those comparing lipid-adjusted serum to adipose tissues. As was the case with gluteal adipose tissue versus lipid-adjusted serum, the comparison of gluteal adipose tissue with non-lipid-adjusted serum for PCB-153, resulted in a poor correlation ($r_{\text{PCB-153}} = 0.04$; data not shown).

Ratios between the various media comparisons are presented in Table 2. The values presented are the arithmetic mean, median, and geometric mean of the ratio of each individual's paired samples. We found the relationships consistent, whether they were of highly abundant compounds (DDT and β -HCH) or in low concentrations (PCB-153 and PCB-180), and whether they were likely recent exposures or not. With the exception of comparisons between non-lipid-adjusted serum and adipose tissues, ratios ranged from 0.4:1 to 1.8:1, with many comparisons having ratios close to 1:1. Among all five compounds, breast and gluteal adipose tissue concentrations versus serum (ng/mL) concentrations ranged from 66.4:1 to 311.1:1. Ratios for each compound measured and

for each comparison did not differ substantially by summary statistic.

When we stratified by younger and older age group and by shorter and longer time of breast-feeding, we found no difference with respect to the correlations or ratios.

Discussion

To our knowledge, this is the first study to use three biological media to compare concentrations of multiple organochlorine compounds via correlation analyses and calculation of ratios across means, medians, and geometric means of five comparisons (serum versus breast, lipid-adjusted serum versus breast, serum versus gluteal, lipid-adjusted serum versus gluteal, and breast versus gluteal), among fasting women, whose samples were taken before treatment. This study confirms previous findings of strong correlations between serum and adipose tissue concentrations of various organochlorines (1-5) and strong correlations between various adipose tissues (6). Distinct from most previous studies however, the women in this population were fasting, before having their blood drawn. We believe that this enabled us to report reliable correlations and ratios not only for lipid-corrected values of serum concentrations (ng/g) but also for serum concentrations not corrected for lipids (ng/mL). We detected ratios close to 1:1 for many of the comparisons among lipid-corrected serum, breast adipose, and gluteal adipose tissue.

We found six previous studies in the literature which investigated the correlations and ratios of organochlorine concentrations in different biological media. Lopez-Carillo et al. (1) measured levels of DDE in both serum and adipose tissue samples from nonfasting, histologically confirmed breast cancer cases (before any treatment) and benign breast disease controls. They found the average of all individual adipose/serum ratios to vary by summary statistic (arithmetic mean and geometric mean) and by lipid-adjusted status of serum, despite case-control status. The ratio of arithmetic means of breast adipose tissue to lipid-adjusted serum DDE levels was 4.2:1, whereas the ratio of geometric means of adipose tissue to lipid-corrected serum DDE levels was 1.1:1. On a wet weight basis, the ratio of arithmetic means was 161:1, whereas the ratio of geometric means was 5.8:1. The authors did not report correlation coefficients. In contrast to the findings of Lopez-Carillo et al. (1), our ratios did not vary by summary statistic measured. The differences between their findings and our findings may be partially explained by their inclusion of nonfasting women. Analogous to their findings however, we report a considerable difference in ratio, depending on whether serum was not adjusted or adjusted for lipids. Stellman et al. (2) compared the concentrations of seven organochlorine pesticides and 14 PCB congeners, measured in blood serum (not adjusted for lipids) and adipose tissue. Serum-breast adipose correlation coefficients were statistically significant for DDE ($r_{p,p'-DDE} = 0.73$), DDT ($r_{p,p'-DDT} = 0.70$), and β -HCH ($r_{\beta-HCH} = 0.58$). The authors reported strong correlations between serum not adjusted for lipids and breast adipose tissue measurements of DDE, DDT, and β -HCH but not for PCB-153 or PCB-180. The authors did not report findings for lipid-adjusted serum. The ratios of mean serum (ng/mL) versus mean breast adipose tissue were similar to ours for DDE, PCB-153, and PCB-180; however, their ratios for the same comparison for DDT and β -HCH were considerably lower than ours (for DDT: Stellman et al., 78.6:1; our study, 215.9:1; for β -HCH: Stellman et al., 27.0:1.0; our study, 215.9:1). The correlation coefficients in our study were generally stronger for all comparisons and all organochlorines measured. A study of 41 mothers who gave birth by cesarean in Nairobi, Kenya found a significant correlation between the levels of total DDT in subcutaneous fat and serum fat ($r = 0.87$), indicating the coherence of DDT in the body (5). Likewise, in a study of the concentrations of organochlorine compounds in blood com-

partments (blood cells and blood serum) and adipose tissues, Mussalo-Rauhamaa (3) found a positive correlation between DDT levels in paired lipid-adjusted serum/adipose tissue samples ($r = 0.74$). His study found consistently higher ratios than we did for lipid-adjusted serum versus breast adipose tissue. For DDE and DDT, he found adipose to lipid-adjusted serum ratios of about 3:1 and 2:1, respectively, and like our findings, these ratios held constant across various summary statistics (arithmetic mean, median). A study by Archibeque-Engel et al. did not find any significant relationship between serum concentrations and tissue residues for most compounds they analyzed; however, they did find strong correlations for p,p' -DDE, and their ratio of lipid-adjusted serum versus adipose tissue concentrations of DDE was 1:1 (4).

In a recently published study, Petreas et al. measured levels of organochlorine pesticides, PCBs, polybrominated diphenyl esters, and dioxins in the breast and abdominal adipose tissues of 21 cases who were undergoing mastectomies with simultaneous breast reconstruction, using abdominal fat tissue (6). It is unclear if the breast cancer cases in this study contributed samples before any treatment. Strong correlations between measurements in both adipose tissues were found. Although concentrations of some chemicals (DDT and β -HCH) were much lower than those detected in our study, we had similar findings for breast versus gluteal adipose tissues. Petreas et al. emphasized that measurements in serum may be problematic due to surges in blood lipids, partitioning of chemicals in various blood components according to its relative solubility, and analytic error (6). However, our study found strong correlations between lipid-adjusted serum concentrations and concentrations in both breast and gluteal adipose tissues.

There was one comparison, PCB-153 gluteal adipose tissue versus serum, for which the correlation was poor. We investigated the data further and found that there was one sample for which the serum measurement relative to both the gluteal and breast adipose tissue measurements was high. We found that when we eliminated this outlier, the correlation coefficient for breast adipose versus serum increased from $r = 0.69$ to $r = 0.92$, and the correlation coefficient for gluteal adipose versus serum increased from $r = -0.03$ to $r = 0.13$, which is still low.

The concentrations of β -HCH detected in this population from Kerala were orders of magnitude higher than what has been reported throughout the breast cancer literature

Table 2. Ratios of means, medians, geometric means between serum, adipose tissue, and gluteal tissue

Organochlorine	Breast (ng/g) versus serum (ng/mL)	Gluteal (ng/g) versus serum (ng/mL)	Breast (ng/g) versus serum* (ng/g lipid)	Gluteal (ng/g) versus serum* (ng/g lipid)	Breast (ng/g) versus gluteal (ng/g)
DDE					
Mean	129.1	175.6	0.7	0.9	0.7
Median	135.5	147.9	0.7	0.7	0.9
Geometric mean	148.1	199.2	0.6	0.8	0.7
DDT					
Mean	215.9	288.8	1.1	1.5	1.1
Median	215.1	251.1	1.0	1.2	1.0
Geometric mean	199.3	268.5	1.0	1.3	0.7
β -HCH					
Mean	215.8	311.1	1.1	1.6	0.7
Median	188.4	283.8	1.0	1.4	0.7
Geometric mean	221.0	273.5	1.1	1.4	0.8
PCB-153					
Mean	144.3	130.8	0.7	0.7	1.1
Median	83.8	114.3	0.4	0.6	0.7
Geometric mean	66.4	95.6	0.4	0.5	0.7
PCB-180					
Mean	152.7	151.0	0.8	0.8	1.01
Median	143.0	162.0	0.6	0.6	0.9
Geometric mean	66.4	95.6	0.4	0.5	0.7

*Lipid adjusted.

published in the last 10 years (Appendix 3). These high levels could be attributed to the widespread use of β -HCH in India and the high concentrations found in food. β -HCH (also called technical-grade benzene hexachloride) has been used in India as an insecticide to control locusts and malaria carrying mosquitoes since the late 1940s, being officially banned in 1997 (17-19). It is the most widely used pesticide in India (20), and it is widely distributed throughout the food chain, especially present in meat and milk (21). It is thought therefore that the average Indian dietary intake of β -HCH exceeds that of the United States and the United Kingdom by 100-fold (20). In 2001, the Indian Council of Medical Research reported levels of β -HCH in human fat samples to range from 1,060 to 16,850 ppb ($\mu = 3,490$ ppb; refs. 22, 23). Our measurements fall into this range ($\mu = 3,119$ ppb); however, we recognize that the CV of 33.5% for β -HCH in our study was rather high. As we expected, in this population the levels of DDT (another insecticide used in India subsequent to the ban in most areas worldwide) were also higher than concentrations found in most of the other studies in the literature. However, levels of DDE, the main metabolite of DDT, are actually lower than concentrations found in other studies throughout the literature. Levels of PCB-180 were lower than the levels reported in all other studies we reviewed in the literature. These findings are consistent with the fact that the region of Kerala is not highly industrialized.

An important consideration when investigating levels of organochlorine compounds is age, because body burden has been found to be strongly associated with it in Western populations. Older women usually have higher concentrations. We stratified the population into older (>45 years) and younger (≤ 45 years) age groups and found that the older women did have higher concentrations in both serum and adipose tissues; however, there were no differences in correlation coefficients or ratios. Another important consideration is breast-feeding, a highly efficient way of lowering one's body burden (16). If breast-feeding preferentially reduces serum levels relative to adipose levels (due to different pharmacokinetics of elimination), this could reduce correlations. For this reason, it is important to ascertain breast-feeding duration and timing in future studies of organochlorines and breast cancer. When we stratified the population into shorter (≤ 48 months) and longer (>48 months) time of breast-feeding, we found that the women who breast-fed for >48 months did have lower concentrations than women who breast-fed up to 48 months; however, there was no difference in correlation coefficients or ratios.

A strength of our study is that we were able to comprehensively investigate the partitioning of organochlorines among three biological media among subjects who were fasting before the collection of all three biological media. Previous studies have not included these features. There is no perfect method to adjust for lipids in serum, and there are various equations that are commonly used to calculate lipid adjustment (15). Of three equations, Eqns. (1) to (3), described by Phillips et al. (15), Eq. (1) accounts for total cholesterol, free cholesterol, triglycerides, and phospholipids; Eq. (2) accounts for total cholesterol and triglycerides; and Eq. (3) accounts for only total cholesterol. Depending on which method is used to lipid adjust, differences between nonfasting and fasting samples may be eliminated to varying degrees. For example, Phillips et al. found that Eq. (1) is the most accurate, Eq. (2) was not as successful in eliminating differences between fasting and nonfasting samples, and Eq. (3) considerably over adjusted. Unless a complete characterization of total serum lipid components is used, the data may not adequately yield comparable results between fasting and nonfasting subjects. We, therefore, consider the measurements of fasting subjects, in conjunction with serum lipid adjustment, to be the most accurate way to measure organochlorines in serum.

Although the subjects in this study were all breast cancer cases, we do not feel that this has biased our results in any way. We did not find any differences between low-grade tumors (stages I and II) and high-grade tumors (stages III and IV) in this population. Additionally, the women in this study were all recently diagnosed breast cancer cases, and biological samples in this study were taken shortly after diagnosis and prior to treatment.

It is important to confirm that measurements of organochlorines in blood serum are an accurate reflection of the distribution of these compounds in breast adipose tissue and gluteal tissue because it is much less invasive to collect a blood sample than a fat sample. In summary, correlations for serum with both breast and gluteal adipose tissues were consistently strong across a range of exposure levels (high or low) and across a variety of compounds (organochlorine pesticides, an organochlorine pesticide metabolite, a PCB congener). These findings further substantiate that blood serum collected from fasting subjects may be used in lieu of other media to accurately reflect the partitioning of a variety of organochlorines in humans. These data indicate that blood serum reflects the present body burden of a range of organochlorines

Appendix A. DDE levels measured in blood serum (unadjusted: $\mu\text{g/mL}$, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature

Author	Period of sample collection	Population	Serum, unadjusted (all converted to ng/mL)			Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean	Mean	Median	Geometric mean
Romieu (24)	1990-1995	Mexico City	17.5	—	—	2,510	—	—	—	—	—
Helszouser (25)	1974	Washington county, MD	13.6	11.1	—	1,920.3	1,668.4	—	—	—	—
Millikan (26)	1993-1996	North Carolina (African American)	8.8	5.7	—	1,690	1,170	—	—	—	—
Helszouser (25)	1989	Washington county, MD	9.7	7	—	1,586.3	1,181.7	—	—	—	—
Rubin (27)	1980-1987	Alaska (Inuit, Aleut, AK native)	9.1	—	—	1,395.7	—	—	—	—	—
Ward (28)	1973-1991	Norway	8.2	—	—	1,260	—	—	—	—	—
Rusieck, 2005	1997	India OCC Study	4.72	3.11	2.82	944.48	619.44	260.23	609.37	421.55	417.66
Millikan (26)	1993-1996	North Carolina (Caucasian)	3.9	2.3	—	760	430	—	—	—	—

(Continued on the following page)

Appendix A. DDE levels measured in blood serum (unadjusted: $\mu\text{g/mL}$, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature (Cont'd)

Author	Period of sample collection	Population	Serum, unadjusted (all converted to ng/mL)			Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean	Mean	Median	Geometric mean
Lopez-Carillo (1)	1994-1996	Mexico	4.1	—	1.1	505.5	—	20.5	—	—	—
Moysich (29)	1986-1991	Western NY	—	—	—	10.8	—	—	—	—	—
Moysich (30)	1986-1991	Western NY	—	—	—	10.8	—	—	—	—	—
Hoyer (31)	1976-1978	Denmark	—	—	—	—	1,196.6	—	—	—	—
Hoyer (32)	1976	Denmark	—	—	—	—	1,182.9	—	—	—	—
Hoyer (31)	1981-1983	Denmark	—	—	—	—	1,168*	—	—	—	—
Laden (33)	1989-1990	Combined (five) U.S. studies	—	6.5	—	—	820	—	—	960	—
Wolff (34)	1987-1992	New York	—	—	7.3	—	—	1,097	—	—	—
Wolff (35)	1994-1996	New York (Caucasian)	—	—	6.4	—	—	1,000	—	—	—
Wolff (35)	1994-1996	New York (African American)	—	—	4.3	—	—	660	—	—	—
Gammon (36)	1996-1997	Long Island	—	—	4.1	—	—	645.7	—	—	—
Zheng (37)	1994-1997	Connecticut	—	—	—	—	—	456.2	—	—	—
Waliszewski (38)	1998, 1991	Mexico	—	—	—	—	—	—	9,970	—	—
Dewailley (39)	1990-1994	Greenland	—	—	—	—	—	—	3,874	—	—
Burgaz (40)	1990-1995	Iran	—	—	—	—	—	—	2,450	—	—
Liljegren (41)	1993-1995	Sweden	—	—	—	—	—	—	1,026	—	—
Mussalo-Rahmanna (42)	1985-1986	Helsinki, Finland	—	—	—	—	—	—	980	—	—
Dewailley (43)	1991-1992	Quebec (fasting)	3.5	—	—	—	—	—	765.3	—	—
Bagga (44)	1995-1996	Kaiser, CA	—	—	—	—	—	—	709	—	—
Stellman (45)	1994-1996	Long Island	—	—	—	—	—	—	374.1	—	—
Olaya-Contreras (46)	1995-1996	Mexico	2.5	0.9	—	—	—	—	—	—	—
Bjerregaard (47)	1994-1996	Greenland	4.8	—	3.7	—	—	—	—	—	—
Schechter (48)	1994	Vietnam	16.7	9.2	—	—	—	—	—	—	—
Kreiger (49)	1964-1971	San Francisco	43.1	—	—	—	—	—	—	—	—
Dorgan (50)	1977-1987	Missouri	—	—	—	—	—	—	—	—	—
Demers (51)	1994-1997	Quebec, Canada	—	—	—	—	—	—	—	—	—
Hoyer (52)	1976-1978	Denmark	—	—	—	—	—	—	—	—	—
Zheng (53)	1994-1997	Connecticut	—	—	—	—	—	—	—	772.8	784.1
Guttes (54)	1993-1994	Germany	—	—	—	—	—	—	—	—	450
Aronson (55)	1995-1997	Ontario, Canada	—	—	—	—	—	—	—	—	596
Della Iacovo (56)	1997-1998	Naples, Italy (fasting)	8.98	—	—	—	—	—	—	—	—
Sala (57)	1994	Spain	9.6	5.2	—	—	—	—	—	—	—

*Middle tertile.

Appendix B. DDT levels measured in blood serum (unadjusted: $\mu\text{g/mL}$, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature

Author	Period of sample collection	Population	Serum, unadjusted (all converted to ng/mL)			Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean	Mean	Median	Geometric mean
Romieu (24)	1990-1995	Mexico City	1.41	—	—	230	—	—	—	—	—
Rusiecki, 2005	1997	Indian OCC study	0.76	0.47	0.62	149.9	97.36	57.22	164.05	101.08	113.58
Ward (28)	1973-1991	Norway	0.9	—	—	137.7	—	—	—	—	—
Lopez-Carillo (1)	1994-1996	Mexico	0.71	—	—	84.53	—	—	—	—	—
Hoyer (31)	1981-1983	Denmark, 1980s	—	—	—	—	45.7*	—	—	—	—
Hoyer (31)	1976-1978	Denmark, 1970s	—	—	—	—	144.2*	—	—	—	—
Hoyer (32)	1976	Denmark	—	—	—	—	141.4*	—	—	—	—
Gammon (36)	1996-1997	Long Island	—	—	0.43	—	—	69.32	—	—	—
Wolff (35)	1994-1996	New York (African American)	—	—	0.17	—	—	30	—	—	—
Wolff (35)	1994-1996	New York (Caucasian)	—	—	0.19	—	—	30	—	—	—
Bagga (44)	1995-1996	Kaiser, CA	—	—	—	—	—	—	267.3	—	—
Stellman (2)	1994-1996	Long Island	0.22	—	—	—	—	—	—	17	—
Della Iacovo (56)	1997-1998	Naples, Italy	1.77	—	—	—	—	—	—	—	—

(Continued on following page)

Appendix B. DDT levels measured in blood serum (unadjusted: µg/mL, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature (Cont'd)

Author	Period of sample collection	Population	Serum, unadjusted (all converted to ng/mL)			Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean	Mean	Median	Geometric mean
Schechter (48)	1994	Vietnam	2.37	—	—	—	—	—	—	—	—
Mussalo-Rauhamaa (3)	1985-1986	Finland	2.8	1.8	—	—	—	—	—	—	—
Stellman (45)	1994-1996	Long Island	—	—	—	—	—	—	—	12.1	—
Zheng (53)	1994-1997	Connecticut	—	49.4	55.6	—	—	—	—	—	—
Aronson (55)	1995-1997	Ontario, Canada	—	—	19.3	—	—	—	—	—	—
Guttes (54)	1993-1994	Germany	—	—	24	—	—	—	—	—	—

*Middle tertile.

Appendix C. β-HCH levels measured in blood serum (unadjusted: µg/mL, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature

Author	Period of sample collection	Population	Serum, adjusted (all converted to ng/mL)			Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g lipid)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean	Mean	Median	Geometric mean
Rusiecki, 2005	1997	India OCC Study	14.45	14.2	8.59	2,876.15	2,818.18	792.99	3,119	2,675	2,751.28
Rubin (27)	1980-1987	Alaska (Inuit, Aleut, AK native)	2.58*	—	—	389.5	—	—	—	—	—
Ward (28)	1973-1991	Norway	0.41	—	—	63.4	—	—	—	—	—
Hoyer (31)	1981-1983	Denmark, 1980s	—	—	—	—	60*	—	—	—	—
Hoyer (31)	1976-1978	Denmark, 1970s	—	—	—	—	119*	—	—	—	—
Hoyer (32)	1976	Denmark	—	—	—	—	118.9*	—	—	—	—
Mussalo-Rahmanna (42)	1985-1986	Finland	—	—	—	—	—	—	80	—	—
Dewailley (43)	1991-1992	Quebec	—	—	—	—	—	—	39.7	—	—
Stellman (2)	1994-1996	Long Island	0.82	—	—	—	—	—	22.2	—	—
Della Iacovo (56)	1997-1998	Naples, Italy	1.49	—	—	—	—	—	—	—	—
Stellman (45)	1994-1996	Long Island	—	—	—	—	—	—	—	15.8	—
Zheng (58)	1994-1997	Connecticut	—	—	—	—	—	—	—	25.2	26.3
Aronson (55)	1995-1997	Ontario, Canada	—	—	—	—	—	—	—	—	41.5
Guttes (54)	1993-1994	Germany	—	—	—	—	—	—	—	—	72

*Middle tertile.

Appendix D. PCB-153 levels measured in blood serum (unadjusted: µg/mL, adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature

Author	Period of sample collection	Population	Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean
Ayotte (59)	1992	(Pooled samples), S. Quebec	33.0	—	—	—	—	—
Ayotte (59)	1992	(Pooled samples), Nunavik	1,270.0	—	—	—	—	—
Liljegren (41)	1993-1995	Sweden	291.0	—	—	—	—	—
Demers (51)	1994-1997	Quebec, Canada	55.6	51.1	—	—	—	—
Demers (60)	1994-1997	Quebec, Canada	51.0	50.7	—	—	—	—
Hoyer (31)	1976-1978	Denmark (1970s)	—	223.4	—	—	—	—
Hoyer (31)	1981-1983	Denmark (1980s)	—	203.6	—	—	—	—
Gammon (36)	1996-1997	Long Island, NY	—	—	153.2	—	—	—
Guttes (54)	1993-1994	Germany	—	—	—	437.0	516.0	—
Aronson (55)	1995-1997	Ontario, Canada	—	—	—	—	—	98.3
Stellman (45)	1994-1996	Long Island	—	—	—	—	63.1	—
Zheng (61)	1994-1997	Connecticut	—	—	—	—	(100.6-153.9)*	—
Rusiecki, 2005	1997	India	16.1	11.4	13.4	11.5	5.0	4.7

*Middle tertile.

Appendix E. PCB-180 levels measured in blood serum (adjusted: ng/g lipid) and breast adipose tissue (ng/g) for controls or total subjects, throughout the literature

Author	Period of sample collection	Population	Serum, adjusted (all converted to ng/g lipid)			Breast adipose tissue (all converted to ng/g)		
			Mean	Median	Geometric mean	Mean	Median	Geometric mean
Ayotte (59)	1992	(Pooled samples), Nunavik	680.0	—	—	—	—	—
Ayotte (59)	1992	(Pooled samples), S. Quebec	22.0	—	—	—	—	—
Liljegren (41)	1993-1995	Sweden	198.0	—	—	—	—	—
Demers (60)	1994-1997	Quebec, Canada	31.1	30.2	—	—	—	—
Hoyer (31)	1976-1978	Denmark (1970s)	—	85.8	—	—	—	—
Hoyer (31)	1981-1983	Denmark (1980s)	—	82.2	—	—	—	—
Gammon (36)	1996-1997	Long Island, NY	—	—	78.6	—	—	—
Guttes (54)	1993-1994	Germany	—	—	—	258.0	259.0	—
Aronson (55)	1995-1997	Ontario, Canada	—	—	—	—	—	65.7
Stellman (45)	1994-1996	Long Island	—	—	—	—	33.7	—
Zheng (61)	1994-1997	Connecticut	—	—	—	—	(75.6-111.3)*	—
Rusiecki, 2005	1997	India	5.8	5.1	13.4	4.6	2.9	4.7

Note: We did not include serum unadjusted for lipids in this table, as there was only one other study which reported this level.

*Middle tertile.

to the same extent as those in adipose tissue, and they support the hypothesis that serum may be collected in lieu of adipose tissue to obtain similar information. However, such measurements are a combination of earlier exposures, which have metabolized slowly and may still persist, and more recent exposures; thus, investigators should use caution when assigning a level as lifetime body burden.

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